

The University of Chicago

CYTOLOGICAL AND TAXONOMIC
STUDIES IN THE GENUS
BRODIAEA

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1941

By

MADELINE PALMER BURBANCK

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CYTOLOGICAL AND TAXONOMIC STUDIES IN THE GENUS BRODIAEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 530

MADELINE PALMER BURBANCK

(WITH TWENTY-NINE FIGURES)

Introduction

Brodiaea is the genus name commonly applied to a group of liliaceous plants which are widespread in the Pacific coast region of western North America. Certain plants native to South America have been included in the genus in the past, but it is probable that the unjointed pedicels and other characters of the South American plants justify their assignment to a different group. The genus *Brodiaea* ranges from British Columbia in the north to Lower California in the south, being especially common on the lower slopes of the Cascade and Sierra Nevada Mountains. Certain species extend as far eastward as Montana, Wyoming, Utah, Nevada, and Arizona. The plants produce seeds but in the majority of cases are more effectively propagated by offsets from the underground corms.

The twenty-five lots of plants, both species and named varieties of species, used in this study were all received under the genus name *Brodiaea*. Although this generic name had been most commonly applied to this group of plants, various subdivisions have been given in the past and again revived in recent publications by HOOVER (4, 5, 6, 7). In the first paper of this series, HOOVER summarizes the problem of synonymy in the genus, and it is not necessary to discuss the subject at length here. JEPSON (8) recognizes the genus *Brodiaea*, which he divides into three subgenera, *Triteleia*, *Hookera*, and *Dichelostemma*. ABRAMS (1) names six genera (*Calliprora*, *Hesperoscordum*, *Triteleia*, *Hookera*, *Dichelostemma*, and *Brevortia*) to cover essentially the same plant material. More recently HOOVER's reports (4, 5, 6, 7), based on extensive field work in the western states, present morphological characters which he considers sufficiently distinct to justify a division of the plants into four genera. These are essentially the same groups as those recognized by JEPSON, with the addition of a new genus, *Triteleiopsis*. Under International Rules the reorganized groups become *Triteleia*, *Dichelostemma*, and *Brodiaea*. Since the plants used in this study bear out HOOVER's classification with respect to morphological characters and cytological observations, the generic and specific names he gives are, for the most part, used in this paper. Table 1 gives the synonymy. Species of *Brodiaea*, *Dichelostemma*, and *Triteleia* were studied, but plants of the monotypic genus *Triteleiopsis* were not available.

Brodiaea has previously been investigated cytologically by only three workers. In a list of plants possessing 10-12 unequal sized chromosomes, MÜLLER (10) in-

TABLE 1
SYNONYMY OF PLANT MATERIAL WITH EQUIVALENT REPORTED
AND OBSERVED CHROMOSOME NUMBERS

AS NAMED BY HOOVER	AS NAMED WHEN RECEIVED (Purdy)	CHROMOSOME NUMBERS		
		OBSERVED		JOHANSEN
		2N	N	
<i>Brodiaea</i>	<i>Brodiaea</i>			
<i>Coronariae</i>				
<i>B. elegans</i>	<i>B. grandiflora</i>	32	16	
<i>B. coronaria</i>	<i>B. grandiflora</i> (Oregon)	42	21	2n = 42
<i>Stellares</i>				n = 21
<i>B. minor</i>	<i>B. purdyi</i>	32	16	
<i>B. minor</i> var. <i>nana</i>	<i>B. minor</i>	12	6	2n = 14
<i>B. stellaris</i>	<i>B. stellaris</i>	12	6	2n = 12
<i>Appendiculatae</i>				
<i>B. californica</i>	<i>B. californica</i> (Blue)	12	6	2n = 10
<i>B. californica</i>	<i>B. californica</i> (Lilac Pink)	12	6	2n = 10
<i>Dichelostemma</i>				
<i>D. pulchellum</i>	<i>B. capitata</i>	18		2n = 72
.....	<i>B. capitata</i> var. <i>multiflora</i>	18		
<i>D. congestum</i>	<i>B. pulchella</i>	36		2n = 36
<i>D. volubile</i>	<i>B. volubilis</i>	18	9	2n = 36
<i>D. ida-maia</i>	<i>B. coccinea</i>	48		n = 20(ca.)
<i>Triteleia</i>				
<i>Eutriteleia</i>				
<i>T. grandiflora</i>	<i>B. douglasii</i>	32		
<i>T. peduncularis</i>	<i>B. eastwoodii</i>	14		
.....	<i>B. peduncularis</i>	28		
<i>T. laxa</i>	<i>B. laxa</i>	28(30)	14	14*
<i>T. laxa</i>	<i>B. laxa</i> (Blue King)	42		
<i>T. laxa</i>	<i>B. candida</i>	48		
<i>T. crocea</i>	<i>B. crocea</i>	16		
<i>Calliprora</i>				
<i>T. ixioides</i> var. <i>scabra</i>	<i>B. ixioides</i> var. <i>splendens</i>	10(11)	5(6)	5*
<i>T. ixioides</i> var. <i>analina</i>	<i>B. ixioides</i> var. <i>erecta</i>	50		
<i>Hesperoscordum</i>				
<i>T. hyacinthina</i>	<i>B. lactea</i>	28		
<i>T. hyacinthina</i>	<i>B. lactea</i> (Oregon)	28		
<i>T. hendersoni</i>	<i>B. hendersoni</i>	32	16*	
<i>T. bridgesii</i>	<i>B. bridgesii</i>	16		

* Microspore counts.

cluded an unnamed species of *Triteleia*. JOHANSEN (9) worked with four species of *Hookera* (*Brodiaea*), four of *Dichelostemma*, and one of *Brevoortia* (*D. ida-maia*). The chromosome numbers he obtained are given in table 1, and in addition to those listed, he observed 30 somatic chromosomes in *D. multiflorum*. SMITH (12) reported the presence of chromosome rings and end-to-end attachment of more

than 2 chromosomes at microsporogenesis in *B. lactea*. Counts revealed about 21–24 pairs of chromosomes.

It is hoped that the present study will help to clarify relationships within the large group *Brodiaea*, through chromosome counts of approximately half the plants included in the genera *Brodiaea*, *Dichelostemma*, and *Triteleia*.

Material and methods

Plants of the twenty-four species and varieties used in this study were obtained from Mr. CARL PURDY, Ukiah, California, with the exception of corms of *B. coronaria*, which were received from Dr. FRANK H. SMITH, Corvallis, Oregon. Additional material of *B. lactea* (*T. hyacinthina*) was received from Dr. D. P. ROGERS of Corvallis, Oregon.

The corms were potted in sandy loam when received in the autumn. Four or five corms of each lot were kept in a cool greenhouse and used for root-tip material. Root tips were cut off as they emerged and were fixed in La Cour's 2 BE. The material was run up through the usual schedule of alcohols, chloroform and alcohol, and imbedded in paraffin. The root tips were cut transversely at 25 μ . The gentian violet-iodine method of staining, with—in some cases—a picric acid modification (13), was used for the root-tip sections. Immediately after being potted, the corms not used for root tips were placed in a coldframe, where they remained for several months during freezing weather. On being brought into a cool greenhouse many of the plants produced flower buds. Anther smears were made of appropriate stages of meiosis, both by the aceto-carmin method and by fixation of smeared anthers in Navashin's solution followed by staining with gentian violet-iodine. A few smears were also secured which showed the mitotic division of the microspore nucleus following meiosis.

As far as possible, some flower buds of each lot of corms were allowed to mature and the flowers used for a check on the identification of the plants. Unless stated to the contrary, fresh and pressed flowers were used for identification of all plant material. The descriptions given by ABRAMS (1), JEPSON (8), and HOOVER (5, 6, 7) were most frequently used, but references to the original descriptions were made occasionally.

A few selfings and cross pollinations were made with plants which bloomed profusely. Seeds were collected from five selfings and from one cross pollination. Several other pollinations produced negative results. No attempt has been made as yet to germinate the seeds obtained.

Unless otherwise stated, all drawings were made at table level with the aid of a camera lucida, using a 15 $\times$ compensating ocular and a 90 $\times$ apochromatic objective, n.a. 1.40, with a yellow-green filter. The magnification is approximately 2000 $\times$.

Identification of plant material and cytological observations

BRODIAEA

Within this genus HOOVER (5) recognizes four sections: Coronariae, Stellares, Appendiculatae, and Filifoliae. It is probable that two species of the Coronariae were employed in this study. The material received from PURDY under the name *B. grandiflora* was identified as *B. elegans* as described by HOOVER. The staminodia were acute, blunt, or irregularly three toothed, and always shorter than the stamens. The diploid chromosome number of this species is 32 (fig. 1), and 16 chromosomes (fig. 1a) were distinguishable in each early telophase I group. One chain of 4 chromosomes was seen at metaphase I (fig. 8). A few plants were selfed and two capsules of seeds obtained.

When the *B. grandiflora* material from Oregon bloomed at the same time as did the plants from PURDY, slight differences in gross morphology of the flowers could be detected. The tube of the Oregon flower was about 1 mm. longer than that of *B. elegans* and somewhat campanulate rather than funnelform; the segments were approximately the same length as those of *B. elegans* or slightly shorter and 1-2 mm. narrower; the staminodia were erect, not involute, tapered to an acute tip, and were the same length as the stamens; the anthers were 7 mm. in length as compared with 8-9 mm. in the material from PURDY. The leaves of the Oregon material were 2 mm. in diameter and were round or only slightly flattened, while those of *B. elegans* were 2-6 mm. wide and flattened. Chromosome counts of the Oregon material show a diploid number of 42 (fig. 2) and 21 chromosomes (fig. 2a) at metaphase II. Bridging was frequently observed in anaphase and telophase of meiosis I.

Although the Oregon material bloomed sparingly, the consistent flower differences, especially the relative lengths of stamens and staminodia and the distinctive chromosome number, suggest that the material is not *B. elegans*, which it resembles, but rather *B. coronaria* or a form of this species. The staminodia did not form a perfect "corona" but were not so far distant from the center of the flower as were those of the California material. A note from Dr. SMITH during the course of this investigation stated that the material which he sent as *B. glandiflora* had been identified as *B. coronaria* by Prof. HELEN M. GILKEY.

In the Stellares section, both root-tip and anther material of *B. minor* var. *nana* and *B. stellaris* showed that $2n=12$ (figs. 3, 5) and $n=6$ (figs. 3a, 5a). A metaphase figure of the microspore division was counted and verified the count of $n=6$ in *B. stellaris* (fig. 5b).

Corms were received from PURDY under the name *B. purdyi*. JEPSON (8) does not mention this name, but both ABRAMS (1) and HOOVER (5) give *Hookera purdyi* and *Brodiaea purdyi* as synonyms for *Brodiaea (Hookera) minor*. Since certain



FIGS. 1-10.—Fig. 1, *B. elegans*, root-tip metaphase. Fig. 1a, same, early telophase I. Fig. 2, *B. coronaria*, root-tip metaphase. Fig. 2a, same, metaphase II. Fig. 3, *B. minor* var. *nana*, root-tip metaphase. Fig. 3a, same, metaphase I. Fig. 4, *B. purdyi*, root-tip metaphase. Fig. 4a, same, anaphase I. Fig. 5, *B. stellaris*, root-tip metaphase. Fig. 5a, same, metaphase I. Fig. 5b, same, microspore division. Fig. 6, *B. californica* (Blue), root-tip metaphase. Fig. 6a, same, metaphase I. Fig. 7, *B. californica* (Lilac Pink), root-tip metaphase. Fig. 7a, same, metaphase I. Figs. 8-10, associations of 4 chromosomes at metaphase I. Fig. 8, *B. elegans*. Fig. 9, *T. hyacinthina* (aceto-carmin smear). Fig. 10, *B. purdyi*.

floral parts of PURDY'S *B. purdyi* were larger than the measurements given by HOOVER for *B. minor*, some doubt existed as to the correct names for the plants under investigation. The differences to be enumerated later leave no doubt that two distinct forms are involved. Since HOOVER separates *B. minor* var. *nana* from the species on the basis of smaller size without giving a complete set of measurements, the name is used here for the smaller plants. Although the other, larger plant is probably HOOVER'S *B. minor*, the name *B. purdyi* is retained in this discussion as an indication that the plants did not conform in every respect to any description given by HOOVER.

In vegetative characters *B. purdyi*, in contrast to *B. minor* var. *nana*, showed more vigorous growth with longer and broader leaves and usually more than one scape from a single corm. Significant differences in length of plant parts are given in table 2. In addition, the segments of *B. purdyi* recurved soon after the flower

TABLE 2
MEASURED DIFFERENCES BETWEEN *B. PURDYI* AND
B. MINOR VAR. *NANA*

PLANT PART	<i>B. PURDYI</i>	<i>B. MINOR</i> VAR. <i>NANA</i>
Scape.....	3-12 cm. (av. 8-9 cm.)	3-13 cm. (av. 6 cm.)
Tube.....	7-8 mm.	8-9 mm.
Segments.....	16-19 mm.	9-12 mm.
Staminodia.....	11 mm.	6-7 mm.
Anthers.....	6 mm.	4 mm.

opened, while those of *B. minor* var. *nana* did not. With increasing age the flowers of *B. purdyi* faded to a bluish violet, with a prominent, dark midvein, while those of *B. minor* var. *nana* darkened and shriveled.

Root-tip cells of *B. purdyi* contain 32 chromosomes (fig. 4). At meiosis I and II the reduced number of 16 (fig. 4a) can be observed. Occasionally there are associations of 3 or 4 chromosomes and one ring of 4 was observed (fig. 10). One plant set seed after being selfed.

The only other species of *Brodiaea* studied was *B. californica*. PURDY offers two color forms of this species, "Lilac Pink" and "Blue." Under greenhouse conditions these color differences were not always noticeable. The leaves of the Lilac Pink plants tended to be wider than those of the Blue. For both lots the mitotic chromosome number was 12 (figs. 6, 7) and the meiotic number 6 (figs. 6a, 7a). At both stages the chromosomes of *B. californica* Lilac Pink were slightly larger than those of the blue form. One of the corms received as *B. californica* Lilac Pink produced white flowers, but no irregularities of chromosome structure or behavior were observed at meiosis.

DICHELOSTEMMA

Of the six species and one variety listed by HOOVER (6) in this genus, chromosome counts were made of four species and one variety. Neither *B. capitata* nor *B. capitata* var. *multiflora*, as received from PURDY, flowered in Chicago. According to synonymy, *B. capitata* is *D. pulchellum*, but a corresponding synonym for the variety "*multiflora*" was not found. In both plants $2n = 18$ (figs. 11, 12).

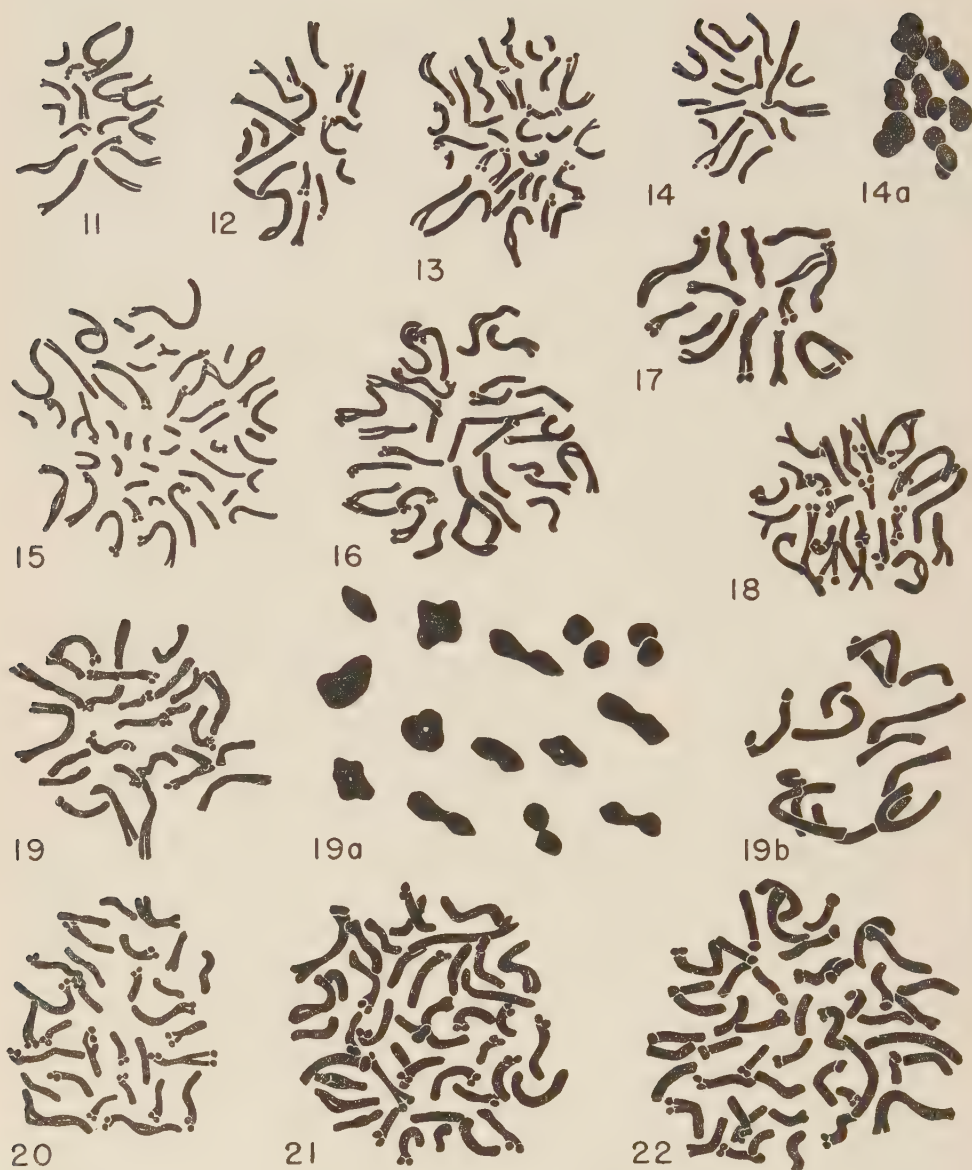
D. congestum has a diploid number of 36 (fig. 13). The few meiotic stages observed did not give a clear picture of the chromosomes, but the number of bivalents appeared to be approximately 18. *D. volubile* had a diploid number of 18 (fig. 14), and 9 bivalents (fig. 14a) were seen at metaphase I. No seeds were set following selfing. *D. ida-maia* has a diploid number of 48 (fig. 15). The large number of chromosomes made it difficult to distinguish individual bivalents at meiosis I, but at metaphase II approximately 24 chromosomes could be seen. One capsule of seed was collected following pollination of *D. volubile* with *D. ida-maia* pollen.

TRITELEIA

On the basis of the attachment and length of the anther filaments, HOOVER (7) divides this genus into three sections, Eutriteleia, Calliprora, and Hesperoscor-dum. First among the plants in the section Eutriteleia, which have the stamens attached alternately in two rows on the perianth, is the type species of the genus *T. grandiflora*. The somatic chromosome number is 32 (fig. 16). Although a few flowers were produced which made it possible to identify the plants, there was not sufficient material for meiotic counts, since the corms tended to split into smaller units rather than to send up flowering scapes.

The second species of the Eutriteleia section is *T. peduncularis*. Some confusion exists about two distinct plants which have been called by this name. According to PURDY (private correspondence with the writer), the plants which he has under the name *B. eastwoodii* have been identified by western taxonomists as *B. peduncularis*. The plants which he carries as *B. peduncularis* are then without a name. This matter will be discussed in greater detail later. Since the plants did not flower, PURDY's determination of two morphologically distinct plants is accepted, and according to HOOVER's nomenclature, *B. eastwoodii* is *T. peduncularis*, and *B. peduncularis* is unnamed. Examination of root-tip material reveals that for *T. peduncularis* $2n = 14$ (fig. 17), and in the unnamed species $2n = 28$ (fig. 18). The leaves of *T. peduncularis* were unique in that the midrib region was triangularly thickened. Between the acute angle of the keel and the upper surface of the leaf there was a space, slightly pithy, which in cross section measured about 2 mm. on each of the three sides.

According to HOOVER (private correspondence and 7), *T. laxa*, as he recognizes it, contains several forms, but no clear-cut separation can be made on the basis of



FIGS. 11-22.—Fig. 11, *D. pulchellum*, root-tip metaphase. Fig. 12, *D. pulchellum* var. “multiflora,” same. Fig. 13, *D. congestum*, same. Fig. 14, *D. volubile*, same. Fig. 14a, *D. volubile*, metaphase I. Fig. 15, *D. ida-maia*, root-tip metaphase. Fig. 16, *T. grandiflora*, same. Fig. 17, *T. peduncularis*, same. Fig. 18, *T.* unnamed, same. Fig. 19, *T. laxa*, same. Fig. 19a, *T. laxa*, metaphase I; bivalents drawn separately. Fig. 19b, same, microspore division. Fig. 20, same, root-tip metaphase with 2 extra chromosomes. Fig. 21, *T. laxa* Blue King, root-tip metaphase. Fig. 22, *T. candida*, same.

morphological characters. Material received from PURDY under the names *B. laxa*, *B. laxa* Blue King, and *B. candida* should all be called *T. laxa*, according to HOOVER. In this study, however, the species names as given by PURDY will be used with the genus name *Triteleia*, since the three plants are somewhat distinct taxonomically and cytologically. As grown in the greenhouse here, *T. laxa* Blue King usually had slightly broader leaves, longer scapes, and flowers of a deeper lavender-blue than did *T. laxa*. The material was rather variable, especially with regard to the horizontal or upright orientation of the flowers, the location of the pistils along the lower side of the flowers or in a central position, and blue or white coloration of the stamens and pollen. *T. laxa* shows $2n=28$ (fig. 19) in root tip and $n=14$ (fig. 19a) at metaphase of meiosis I and the microspore division (fig. 19b). One plant contained 30 chromosomes (fig. 20) in root-tip cells. The root-tip material of *T. laxa* Blue King shows $2n=42$ (fig. 21). The massing of the large number made accurate counting of meiotic chromosomes impossible, but in a few cells two groups of approximately 21 chromosomes each could be seen at anaphase I. At diakinesis there appeared to be about 19–23 bivalents and 3–5 univalents, with very few chiasmata evident. The haploid number of 14 was seen at meiosis in at least one plant labeled *T. laxa* Blue King and in a microspore division figure from a similar plant where the expected number would be 21. Enlargement of the ovaries followed self-pollination in both *T. laxa* and *T. laxa* Blue King, but no seeds were produced.

The plants of *T. candida* differ from those of *T. laxa* in several respects. The flowers were produced 1–2 months earlier than those of *T. laxa*; they were slightly larger, and the leaves were wider and a more yellow green, with a succulent appearance. This was not caused by some local difference in microclimate because the plants were all treated in the same way, and the differences were apparent both winters that the plants were grown. The morphological characters of *T. candida* flowers were essentially the same as those of *T. laxa*, except for the smaller size of the anthers. PURDY (private communication) maintains that *T. candida* and *T. laxa* can be distinguished by the angled pedicels and horizontal flowers of *T. candida* and by the straight pedicels of *T. laxa*, which are never angled at the base of the tube. Since this information was received from PURDY after the *T. candida* flowers had ceased blooming, fresh material could not be examined, and pressed specimens were unsatisfactory on this point. As already stated, however, some flowers of both *T. laxa* and *T. laxa* Blue King formed an angle with the pedicel, so the distinction does not always hold true.

In *T. candida* $2n=48$ (fig. 22). Again meiotic counts were difficult, but about 22–24 bivalents could be seen in several cases. Some of the configurations at metaphase I suggested multiple associations of chromosomes, but the material requires further study. Two capsules of seed were collected following self-pollination.

T. crocea, another member of the *Eutriteleia* section, had a somatic number of 16 (fig. 23). Since these plants did not bloom, no meiotic counts were possible, and PURDY's determination of the species was accepted.

The *Calliprora* section includes those plants in which the anther filaments are alternately long and short but all attached at the same level. The varieties of



FIGS. 23-29.—Fig. 23, *T. crocea*, root-tip metaphase. Fig. 24, *T. ixioideis scabra*, same. Fig. 24a, *T. ixioideis scabra*, metaphase I. Fig. 24b, same, microspore division (aceto-carmines smear). Fig. 25, same, root-tip metaphase with 1 extra chromosome. Fig. 25a, same, metaphase I with fragment. Fig. 26, *T. ixioideis* var. *analina*, root-tip metaphase. Fig. 27, *T. hyacinthina*, same. Fig. 28, *T. bridgesii*, same. Fig. 28a, *T. bridgesii*, metaphase I. Fig. 29, *T. hendersonii*, root-tip metaphase. Fig. 29a, same, microspore division.

B. ixioideis received from PURDY correspond with the two varieties of *T. ixioideis* which HOOVER (7) places in this group, *T. ixioideis* var. *scabra* and *T. ixioideis* var. *analina*. Three of the four plants from which root tips were collected showed 10 somatic chromosomes (fig. 24) in *T. ixioideis* var. *scabra*. The fourth plant had 11 chromosomes (fig. 25), which exhibited greater extremes in length than did those of the "normal" complement of 10. Meiotic counts from two out of about twelve plants examined revealed an extra chromosome or chromosome fragment at meta-

phase I (fig. 25a) in addition to the normal five pairs (fig. 24a) characteristic of the majority of plants. The extra chromatic material was in the form of a small circular mass. It was never seen associated with a bivalent and frequently was at some distance from the group of bivalents. If present in a single cell, the fragment was present in all cells on the slide in which individual chromosomes or bivalents were distinguishable. At anaphase I and telophase I the fragment appeared capable of going undisturbed to one of the daughter nuclei. Frequently the fragment became isolated in the cytoplasm or remained between the two masses of cytoplasm which rounded up around each nucleus preliminary to the formation of a cell wall between the daughter cells. None of the material obtained showed the fate of the fragment in meiosis II. The reduced number of five chromosomes was observed in one microspore (fig. 24b). Two plants set seed following selfing.

T. ixiioides var. *analina* has a somatic chromosome complement of 50 (fig. 26). Meiotic stages in which the chromosomes could be counted were not obtained. In a few cells there was a small circular mass of chromatin separate from the mass of metaphase I bivalents. At later stages there was very rarely a "fragment" associated with one or both of the masses of chromosomes at telophase I and metaphase II. Attempted crosses between the two varieties with *T. ixiioides* var. *analina* as the pollen parent were unsuccessful.

Chromosome counts were made of three species included in the section *Hesperoscordum*, which contains plants with equal stamens. The material of *T. hyacinthina* from Oregon was identical with that obtained from PURDY. Both had a somatic number of 28 (fig. 27). The plants did not flower profusely, and the meiotic material suggests 14 bivalents at metaphase I and 14 in each chromosome group at metaphase II, but the determination could not be made positively. A ring of four chromosomes (fig. 9) was seen in two separate cells.

T. hendersoni has a diploid number of 32 (fig. 29). No meiotic material was obtained, but the reduced number of 16 was observed at metaphase of the microspore division (fig. 29a). In the absence of any mature flowers, the species determination of PURDY is accepted.

In *T. bridgesii* $2n = 16$ (fig. 28). Eight bivalents were observed at metaphase I (fig. 28a). Self-pollination resulted in enlarged ovaries, but no seeds were matured.

Discussion

The preceding observations indicate that the plants commonly known as Brodiaeas comprise a far from homogeneous group. Taxonomically three divisions can be recognized. Cytologically there might be postulated five groups, with basic numbers of 5, 6, 7, 8, and 9, since the diploid numbers of 10, 12, 14, 16, and 18 occur as well as multiples of these numbers. Grouped according to known chromosome numbers, the lines of the natural divisions as outlined by taxonomists would

be entirely obscured. A more fruitful—and in this case perfectly justifiable—approach to the problem is to show that each of the three genera, *Brodiaea*, *Dichelostemma*, and *Trileleia*, comprises a cytological as well as a taxonomic unit. It is unfortunate that none of the species studied have trabants or other means of ready identification of individual chromosomes. Even the centromere cannot always be located. Comparative lengths of chromosomes are helpful when cells can be found in which the chromosomes all lie in the same plane throughout their length.

Within the genus *Brodiaea*, as used in the limited sense, there are found three somatic numbers, 12, 32, and 42. Six, the gametic number of three of the six plants examined, may be considered the basic number. The similarity of the chromosomes of *B. minor* var. *nana*, *B. stellaris*, and *B. californica* is clearly seen in figures 3 and 5 7. In each plant the shortest chromosome has an approximately subterminal centromere and the longest chromosome a median constriction, indicating the position of the centromere. Likewise each of the other four chromosomes in *B. minor* var. *nana* has its counterpart in *B. stellaris* and *B. californica*. Although each chromosome of *B. californica* is larger than the similar one in the other two species, the straight progression from shortest to longest is relatively the same for all three species. This indicates a close relationship between the species.

Plants received as *B. purdyi*, $2n = 32$, may be derived from a hexaploid variety of a *B. minor* var. *nana* plant containing 36 chromosomes. A theory such as the "dislocation" hypothesis of NAVASHIN (11) could account for the change from 36 to 32 somatic chromosomes. The observation of meiotic irregularities in material of *B. purdyi* lends support to a theory which involves changes in chromosome number and morphology. It is impossible to recognize the 6 basic chromosomes and determine the degree of "ploidy" by a critical examination of the somatic or meiotic cells of *B. purdyi*, but the general range of length is similar to that found in the plants in which $2n = 12$.

The chromosome numbers of *B. elegans*, $2n = 32$, and *B. coronaria*, $2n = 42$, do not suggest a close relationship between these species and those of the Stellares and Appendiculatae, although such a relationship may have existed at one time. *B. coronaria* could be considered a heptaploid, but the number for *B. elegans* is not a multiple of 6. It is possible that polyploidy could have produced two plants with counts of 36 and 48 from plants in which $2n = 12$. Subsequent additions and deletions of whole chromosomes or parts could then have brought about the evolution of the present numbers of 32 and 42. The quadrivalent association observed in *B. elegans* indicates that a reciprocal translocation has occurred. Such a hypothesis of the origin of the species does not correlate with HOOVER'S statement (5) that the plants included in the Coronariae are the most primitive from the

standpoint of morphology. The facts, however, that *B. elegans* and *B. coronaria* have been confused by many taxonomists and put under one species, as may even be the case for the plants here studied, and that intermediate forms have been reported near the northern and southern limits of the range of *B. elegans*, suggest that here may be a group of plants in which chromosome number can be of aid in species delimitation. The species may be in the process of evolution through the development of ecotypes and ecospecies. The fact that *B. elegans* set seed indicates that these plants have at least reached an equilibrium which allows normal sexual reproduction, if the seeds produced are viable. The frequent occurrence of bridging in *B. coronaria* suggests that this species has not attained a similar degree of stability. Duplication of a set of 6 chromosomes cannot be identified; but as in *B. purdyi*, the general range in length and width of the chromosomes is similar to that in the other species of the genus.

JOHANSEN (9) previously reported chromosome counts for four species of *Hookera*, which is synonymous with *Brodiaea* as used by HOOVER (4). His $2n = 12$ for *B. stellaris* agrees with the present findings. His drawing of a root-tip cell of *B. californica* suggests that perhaps 2 of the longest chromosomes may in reality be 4. Such an interpretation would then give a somatic count of 12 as reported here. Conversely, 2 of the chromosomes of *B. minor* as drawn by JOHANSEN would need to be joined to 2 others to agree with the present count of 12. Such corrections are suggested on the basis of very clear mitotic and meiotic figures. The haploid and diploid numbers of 21 and 42, respectively, reported by JOHANSEN for *Hookera coronaria*, suggest that the material tentatively named *B. coronaria* in this study is indeed that species. Sufficient meiotic material was not available for JOHANSEN to observe the presence or absence of irregularities in chromosome behavior in *B. coronaria*.

Dichelostemma is the most homogeneous genus with respect to chromosome number. *D. pulchellum*, a variety of this species, and *D. volubile* each have a somatic number of 18. Nine bivalents could be clearly distinguished at metaphase I in *D. volubile*. *D. congestum*, which has 36 chromosomes, is then a tetraploid or a hexaploid, depending upon the point of view. On the basis of these four plants alone, and bearing in mind that they are assigned to a separate genus, it would be supposed that the basic number is 9 and that *D. congestum* is a tetraploid. Added to this evidence is that of the production of seed by *D. pulchellum*, which would be more usual in a diploid than in a triploid. On the other hand, *D. ida-maia*, with a number of $2n = 48$, is also included in the genus. Forty-eight is a multiple of 6 but not of 9. It has been suggested by many that, because of the peculiar type of flower, *D. ida-maia* should be assigned to a separate genus. There are, however, certain morphological similarities to the members of the genus *Dichelostemma*, and if it is true that *D. venustum* is a hybrid of *D. volubile* and

D. ida-maia, the latter would be expected to belong to the same genus rather than to a separate one. In this work several flowers of *D. volubile* were pollinated with *D. ida-maia* pollen, and two somewhat shriveled seeds were produced. Whether or not they are capable of germination has not yet been determined.

A hypothetical solution of the relationships of the plants included in *Dichelostemma* would be that far back in the beginning of the *Brodiaea* complex, 6 was the basic number. By various means one group of plants may have evolved with 9 as a secondarily basic number. Paralleling the duplication of certain chromosomes to give the diploid number of 18, an octoploid with 48 chromosomes may have arisen which in certain respects had a genetic make-up similar to that of the $2n = 18$ plant. Thus gross morphological characters might be similar and chromosome complements be sufficiently alike to allow crossing. It would be of interest to know the chromosome number of *D. venustum*.

JOHANSEN (9) counted the chromosomes of five species in this genus, one of which, *D. multiflorum*, was not obtained for this study. The reported $2n$ number of 30 might be considered to strengthen the hypothesis of 6 as a basic number. The counts of three other species by JOHANSEN agree in the case of *D. congestum* but are multiples of 18 for *D. volubile* and *D. pulchellum* (table 1). Such variations suggest the existence of polyploid races within a species which are indistinguishable in general morphology. JOHANSEN counted about 20 haploid chromosomes in meiosis in the microsporocytes of *Brevoortia* (*D. ida-maia*) and reported that this may be a plant with oscillating chromosome numbers. Although there was some difficulty in counting the chromosomes of *D. ida-maia* for this study, it was attributed to the fact that several very small chromosomes were easily obscured by the larger ones rather than to an actual variation in number. JOHANSEN mentions this striking variation in size. *D. congestum* likewise has some very small chromosomes. These short chromosomes, and the fact that all the species included in *Dichelostemma* tend to have much smaller chromosome diameters than do those of the other genera, further justify the separation of this group into a distinct genus.

Cytologically the genus *Triteleia* is distinguished by the variation in chromosome numbers among the species and the high proportion of species and varieties with meiotic irregularities. In addition, *T. ixioides* var. *scabra* and *T. laxa* plants contained individuals with chromosome numbers higher than the number in the majority of plants. This aneuploid condition is similar to that found by BEAL (2) in the Mariposa section of *Calochortus*. Somatic chromosome numbers of *Triteleia* include 10 (11), 14, 16, 28 (30), 32, 42, 48, and 50. Three groups can be recognized: (a) those with 10 (11) and the decaploid 50; (b) those with a diploid number of 14, the tetraploids with 28, and the hexaploid with 42; (c) those with a diploid number of 16 and the tetraploids with 32. The species with a diploid number of 48 could be a hexaploid in the third group or belong to a distinct group of

which the basic number was 6. The material examined gave no cytological clue as to possible relationships. Table 1 shows that the sections set forth by HOOVER (7) and the preceding three groups do not include the same plants. Further cytological and taxonomic work on the genus is needed to reconcile these differences.

No basic number for *Triteleia* can be suggested on the basis of present findings. Multiples of 7 and 8 occur in equal numbers. The presence of rings of 4 chromosomes in *T. hyacinthina*, rings of 4 and 6 chromosomes reported by SMITH (12) for *T. hyacinthina* plants with probably twice the chromosome number reported here, fragments in the two varieties of *T. ixioides*, the paucity of chiasmata and the presence of univalents in the *T. laxa* Blue King material, and the early separation of the small chromosomes at meiosis in the *T. laxa* material, all suggest that chromosome changes have played and are continuing to play a significant role in the evolution of species of *Triteleia*. No wonder it is a difficult group taxonomically. It is even possible that 6 was again the original number and other numbers have been derived from a diploid plant with 12 chromosomes. The mechanism for such changes has been outlined by NAVASHIN (11) and applied by BEAL (2) in his discussion of the Mariposa section of *Calochortus*.

The foregoing discussion of cytological findings has demonstrated that chromosome counts may be used to substantiate the division of *Brodiaea* into three genera. Each group is characterized by a separate basic number or by the absence of such a number. It is realized that the evidence is not conclusive, that on the basis of chromosome number alone, *B. elegans* and *B. coronaria* could be assigned to the *Triteleia* group or even all the plants considered a part of a single genus complex, but combined cytological and taxonomic findings indicate that the division into three groups is justified.

While the chromosome counts agree with taxonomic classification in general, this study indicates the need for taxonomic reexamination of certain species. First in *Brodiaea*, chromosome counts would indicate that *B. nana* var. *minor* is the species ($2n=12$) and that the material received as *B. purdyi* ($2n=32$) is a variety derived from the plants with a lower count. More vigorous growth as noted in *B. purdyi* is frequently associated with polyploidy. The presence of a ring of 4 chromosomes and a chain of 3 at meiosis I suggests polyploidy and chromosome rearrangement, including reciprocal translocation. HOOVER, in a private communication to the writer, suggested that the material which PURDY carries as *B. minor* may be *B. minor* var. *nana*. He further stated that EASTWOOD, who originally described *B. purdyi*, has seen the type specimen of *B. minor* in England and finds the two identical.¹ It then remains to re-examine the question of whether

¹ It is also interesting to note that the *B. purdyi* material grown here corresponds more closely with EASTWOOD's (3) original description of the species than with HOOVER's (5) description of *B. minor*. EASTWOOD gives the staminodia length as 13 mm. in contrast to 7-10 mm. and further states that the perianth segments spread widely, recurve, and possess a dark midvein.

B. minor and *B. minor* var. *nana* are a species and a variety or whether they each deserve specific rank. The difference in chromosome number lends support to the latter view, as do also the differences in the flowers as given earlier in this paper. A knowledge of the chromosome numbers of the forms reported by HOOVER (5) which are intermediate between *B. minor* and *B. minor* var. *nana* might be critical for a solution of the problem.

Taxonomic and cytological findings seem to be in agreement within the genus *Dichelostemma*. This is not so completely true in *Triteleia*. Chromosome counts may be a definite aid in the solution of the status of *B. peduncularis* and *B. eastwoodii*. The latter has never been "adequately published under existing rules of nomenclature" (HOOVER, private communication), and for that reason is not listed anywhere as a synonym by him. In the following discussion, however, *B. peduncularis* and *B. eastwoodii* will be used for the sake of clarity to designate the plants received from PURDY under those names. The diploid number of *B. eastwoodii* is 14 and that of *B. peduncularis* is 28. This suggests that the latter is a tetraploid which has arisen from *B. eastwoodii*. The greater length of peduncle reported by PURDY would further substantiate the theory. The isolated and limited habitat, supposedly inaccessible to early botanists, in which *B. peduncularis* was found by PURDY, may indicate that it is of recent origin and has not yet extended its range. Whether it was found by earlier botanists is questionable. PURDY maintains that his *B. peduncularis* more nearly resembles the plant described by WATSON than does the plant for which the description was made, PURDY's *B. eastwoodii*. In this he is supported by Mr. BAKER of Santa Rosa Junior College. Both EASTWOOD and HOOVER maintain that PURDY's *B. eastwoodii* is the true and original *B. peduncularis* as described by WATSON. PURDY suggests that perhaps type localities in some of the older collections are inaccurate, and that his *B. peduncularis* might have been collected and described as such. Later collectors might then have collected PURDY's *B. eastwoodii* from the incorrectly given type locality of *B. peduncularis* and assumed the material to be *B. peduncularis* without examining it critically. Whatever is decided concerning the names and taxonomic classification of the two plants, the chromosome numbers indicate that they are closely related and that "*B. peduncularis*" could be a tetraploid derived from "*B. eastwoodii*."²

Observations made upon the plants received from PURDY under the specific and varietal names *T. laxa*, *T. laxa* Blue King, and *T. candida* indicate that here, indeed, are two species and a variety or form of one of the species rather than a single species, *T. laxa*, as described by HOOVER. *T. laxa* has a haploid number of 14 and diploid number of 28. The observance of 30 somatic chromosomes in the

² Private communications from PURDY are the source of much of the information given in this paragraph.

roots of only one plant indicates that this number is the exception and not the rule. The presence of 42 somatic chromosomes in *T. laxa* Blue King suggests that this is a hexaploid form of the *T. laxa* material. As the plants flowered in the greenhouse, those of *T. laxa* Blue King were darker in color, the perianth segments did not spread so widely, and the leaves were slightly wider than those of *T. laxa*. In other characters, however, such as angle of attachment of the flowers, color of the stamens, and perhaps even chromosome number, the *T. laxa* Blue King plants were variable and intergraded with *T. laxa*. Such evidence indicates that *T. laxa* is a variable species, and that while chromosome counts may give a basis for separation into varieties, consistent morphological characters may not exist to make the separation possible.

Plants studied as *T. candida*, however, have both morphological and cytological characters which distinguish them from the *T. laxa* complex. The somatic number is 48, which is not an exact multiple of 7 as are the counts for *T. laxa*. The anthers of *T. candida* are only 2 mm. in length, while those of *T. laxa* are 3-4 mm. *T. candida* finished blooming before *T. laxa* had opened a single flower. The leaves of *T. candida* were yellowish green and 16 mm. wide; those of *T. laxa* were dark green and 5-10 mm. wide; those of *T. laxa* Blue King were 7-15 mm. wide. It is debatable whether "*candida*" is the correct specific name to use, since the flowers of the plant originally described as *T. candida* were white, but the need for separation from *T. laxa* is indicated.

The presence of an extra chromosome in the root-tip cells of one plant of *T. ixiioides* var. *scabra* does not alter the fact that 10 is considered the typical somatic number. The fact that an extra fragment is present at meiosis lends interest to the situation. Figures 24 and 25 indicate that the increased number is not merely the result of the duplication of a single chromosome, nor could there have been a loss of a single chromosome from a complement of 12. The normal complement is made up of a pair of short chromosomes with a subterminal centromere, 4 chromosomes of medium length with submedian centromeres, and 4 long chromosomes—2 with a submedian centromere and 2 with an approximately median centromere. In the complement with an extra chromosome there are 4 long chromosomes and 4 of medium length, and in addition an extra long chromosome, a shorter than normal chromosome, and a chromosome of about the same length as the shortest in the normal complement. Such a situation could have arisen through duplication of a medium length or a small chromosome which subsequently became fragmented. One small fragment may have become attached to an end of one of the long chromosomes with a median centromere. Another fragment may have become attached to one of the shortest chromosomes. It is supposed that meiotic figures with an extra chromatin mass at metaphase I occurred in plants with a somatic number of 11, but there are no records to prove

the assumption. If the "fragment" did arise through duplication and fragmentation, it is strange that it was never observed associated at meiosis with one or both of the chromosomes of which it was originally a duplicate. Its small size is the most probable contributing cause. If the fragment possesses a centromere, it could be carried regularly through the process of sexual reproduction and be perpetuated. Also through vegetative propagation an irregularity of chromosome number could be retained in ever-increasing numbers of plants, regardless of whether or not they were capable of sexual reproduction. If the change had occurred far back in the history of the species, subsequent genic changes could account for the absence of multiple associations. This explanation of the presence of a chromosome fragment is largely hypothetical, but it is an application of NAVA-SHIN's dislocation hypothesis (11) and is based on the known facts that trisomic plants may arise and that parts of chromosomes may be translocated to non-homologous chromosomes.

Summary

1. The chromosome numbers of twenty-four species and varieties of *Brodiaea* have been investigated.

2. Somatic numbers of 10 (11), 12, 14, 16, 18, 28, 28 (30), 32, 36, 42, 48, and 50 chromosomes were observed. An incomplete series of corresponding gametic numbers includes 5 (6), 6, 8, 9, 14, 16, and 21.

3. Taxonomic and cytological findings seem to justify the separation of the material examined into three genera, *Brodiaea*, *Dichelostemma*, and *Triteleia*. It is suggested that the basic number for *Brodiaea* is 6, and for *Dichelostemma* the secondarily basic number 9, derived from 6; the variation of numbers in *Triteleia* is too great to postulate any one basic number.

4. The need for reconsideration of certain specific delimitations is suggested on the basis of cytological and taxonomic observations.

5. Variation of chromosome number is reported for two plants and an explanation for one of the variations offered on the basis of duplication of a chromosome and subsequent dislocation.

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LITERATURE CITED

1. ABRAMS, LEROY, An illustrated flora of the Pacific states. I. Stanford. 1923.
2. BEAL, J. M., Cytological studies in relation to the classification of the genus *Calochortus*. BOT. GAZ. 100:528-547. 1939.
3. EASTWOOD, ALICE, Descriptions of some new species of Californian plants. Proc. Cal. Acad. Sci. (II) 6:422-430. 1896.
4. HOOVER, R. F., A definition of the genus *Brodiaea*. Bull. Torr. Bot. Club 66:161-166. 1939.
5. ———, A revision of the genus *Brodiaea*. Amer. Mid. Nat. 22:551-574. 1939.
6. ———, The genus *Dichelostemma*. Amer. Mid. Nat. 24:463-476. 1940.
7. ———, A systematic study of *Triteleia*. Amer. Mid. Nat. 25:73-100. 1941.
8. JEPSON, W. L., A manual of the flowering plants of California. Berkeley. 1925.
9. JOHANSEN, D. A., The chromosomes of the California Liliaceae. I. Amer. Jour. Bot. 19: 779-783. 1932.
10. MÜLLER, H. A. C., Kernstudien an Pflanzen. Archiv. Zellforsch. 8:1-51. 1912.
11. NAVASHIN, M., The dislocation hypothesis of evolution of chromosome numbers. Zeitschr. Ind. Abst. Vererb. 63:224-231. 1932.
12. SMITH, F. H., Preliminary studies of chromosome rings in *Brodiaea lactea*. Proc. Nat. Acad. Sci. 19:605-609. 1933.
13. ———, The use of picric acid with the gram stain in plant cytology. Stain Technol. 9: 95-96. 1934.

